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MICROBIOLOGICAL BURDEN ON THE SURFACES OF THE AIMP SPACECRAFT

PART 4

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Laboratory for Atmospheric and Biological Sciences

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SUMMARY

The objective of this study was to reduce the microbiological contamination on the AIMP spacecraft to the lowest possible level, to prevent gross contamination of the lunar surface and to determine the degree of microbiological contamination on a typical spacecraft before and after decontamination. Results show that a decontamination program can reduce the microbiological burden by at least 2 logs.

PART 4

TABLE OF CONTENTS

	<u>Page</u>
SUMMARY	iii
INTRODUCTION.....	1
MATERIALS AND METHODS.....	2
RESULTS.....	4
DISCUSSION.....	7
REFERENCES	12

TABLES

<u>Table</u>	<u>Page</u>
1 Parts of the AIMP Spacecraft Sampled for Microbial Contamination.....	2
2 Microbial Contamination of AIMP Flight Spacecraft During Assembly	4
3 Determination of the Total Microbial Burden of the AIMP Flight Spacecraft After Decontamination.....	5
4 Decontamination of Solar Paddles with Methyl Ethyl Ketone	6
5 Microbial Contamination in the Air of Laminar Flow Clean Rooms Housing the AIMP Spacecraft	8
6 Microbial Contamination in the Air of Laminar Flow Clean Rooms Over a 7-Hour Period.....	9
7 Microbial Fallout on Stainless Steel	10

MICROLOGICAL BURDEN ON THE SURFACES OF THE AIMP SPACECRAFT

PART 4

INTRODUCTION

The NASA Planetary Quarantine Office requires the sterilization of all planet-bound spacecraft. Although this requirement does not affect lunar landing spacecraft, decontamination is required for lunar probes. The AIMP spacecraft was decontaminated in compliance with the NASA Spacecraft Decontamination Policy, stated in Management Manual 4-4-1(7), for the decontamination of lunar landing hardware.

The two primary reasons for this policy are:

- Contamination of a planet with terrestrial microorganisms could frustrate efforts to demonstrate the existence of extra-terrestrial life.
- Overgrowth of a planet with terrestrial microorganisms might alter indigenous extraterrestrial life.

Although a decontamination program will reduce the number of microorganisms to a significant degree, it will not produce a sterile spacecraft. The only acceptable method of sterilization at the present time is exposure of the spacecraft to a given dry-heat cycle. Both the temperature and the duration of exposure to dry heat must ensure that the probability of contaminating a planetary body is not significantly greater than 10^{-4} . Decontaminating agents (alcohol, ethylene oxide, formaldehyde, peracetic acid, beta propiolactone and others) can aid significantly in a sterilization program by reducing the microbiological contamination to a level which will ensure that a given sterilization cycle is effective. The degree to which contamination can be reduced by the use of chemicals must be known to intelligently plan a sterilization cycle.

After completion of the assembly, testing, and decontamination of the AIMP spacecraft at Cape Kennedy, the AIMP spacecraft was successfully launched on July 1 into the highest earth orbit ever achieved. The AIMP was originally intended to orbit the moon and to conduct

scientific investigations from its "anchored" lunar orbit. Although AIMP did not achieve a lunar orbit, it will produce significant scientific results.

The purpose of the decontamination procedure reported here was to reduce the microbiological contamination on the AIMP to the lowest possible level, to prevent gross contamination of the lunar surface if the spacecraft should impact on the moon. The information obtained in the process of decontamination will be of great use in future sterilization programs.

MATERIALS AND METHODS

Table 1 lists the surfaces of the spacecraft which were decontaminated and sampled. The techniques, sampling devices, decontaminating agent and microbiological processing have been previously described (1,2,3).

Table 1

Parts of the AIMP Spacecraft Sampled for Microbial Contamination

Assembly Phase	Area	Nomenclature	Surface Area (sq.in.)	Area Sampled (sq.in.)
7 (6-9-66 to 6-29-66)	C	Top of module stack	328	16
		Front face of stack	460	16
		Inner surface of cover	1430	28
	D	Sun shield plate	34	4
		Spring seat assembly fly away plate	40	4
		Motor adaptor	40	4
		*Circular thermal blanket	3600	4
		Lower motor ring and sphere	89	4
		Retromotor case	490	12
		*Main thermal blanket	39,600	16
	E	Top cover	1369	32
		Solar paddles	5600	48
		Platform lower surface	400	12
		Boom body	485	8

*Sterilized

Clean Rooms

The laminar crossflow clean room monitored was a class 10,000 and is operated by Pan American Airways. The room was 82 feet long, 40 feet wide, and 43 feet high. The laminar downflow room was the same as previously described (3).

Microbial Fallout

Microbial fallout on stainless steel strips was determined by Jerald Tritz, U.S. Public Health Service, Cape Kennedy, Florida. Trays of stainless steel strips (1 by 2 inches, #4 finish) were positioned on one side of the crossflow clean room, upstream and downstream of personnel and midway between the two locations. One tray was also placed in the downflow clean room. Six strips were recovered once a week from each location and assayed for aerobic and anaerobic vegetative and spore cells.

Air Sampling

The slit samplers (Reynier and Sons, Chicago, Illinois) and techniques have been previously described (3). The air samplers were positioned upstream (3 feet from air inlet filters), downstream (3 feet in front of air exhaust filters), and in the downflow room. All samplers were placed at approximately bench top level (3 feet above floor). One-hour samples were taken in the morning and afternoon, except that on two days air samples were collected every hour for 7 hours.

Thermal Blankets

The main and circular thermal blankets were sterilized by dry heat at 135°C for 24 hours. The blankets are multilayered (23 layers). Total surface area of the main thermal blanket is 269 square feet; total surface area of the circular thermal blanket is 24 sq. ft. Surface areas of the blankets and all other surfaces of the spacecraft were determined by the Mechanical Systems Branch, Goddard Space Flight Center.

Solar Panels

The solar panels were not decontaminated in the usual way, with isopropyl alcohol. The paddles and solar cells were cleaned by personnel from the Space Power Technology Branch, Goddard Space Flight Center, with methyl ethyl ketone (MEK) applied with cotton swabs. This procedure was necessary because of the physical properties and delicate nature of the solar cells. After cleaning, the paddles were placed in a metal container which was cleaned and

decontaminated with Wescodyne (75 ppm iodine) and transported from the clean room to the gantry. Three swab samples were taken from each paddle immediately after installation and just before the spacecraft was enclosed by the fairing.

Internal Components

The microbiological contamination of the internal components (resistors, capacitors, transistors, diodes, etc.) had been previously determined (4) and compiled by the Mechanical Systems Branch.

RESULTS

Table 2 lists the microbiological contamination detected on surfaces of the AIMP flight spacecraft during the 7 phases of the assembly. The seventh and last phase of the assembly took place at

Table 2
Microbial Contamination of
AIMP Flight Spacecraft During Assembly

Assembly Phase	Date	Viable Organisms per Square Foot	
		Before Decontamination	After Decontamination
1	11-30-65	21,174	563
2	12-13-65	22,388	230
3	12-23-65	1,000	75
4	1-3-66	2,125	90
5	2-7-66	1,299	219
6	5-2-66 - 5-18-66	416	33
7	6-9-66 - 6-29-66	1,134	94 (1)
Ave./ft ²		7,077	186
Total contamination per spacecraft		3,722,502	268,336 (2)

(1)Excluding solar panels

(2)See Table 3 for calculation.

Cape Kennedy. The number of microorganisms (94 per square foot) after decontamination in assembly phase seven does not include the contamination detected on the four solar paddles.

Table 3 shows the effect of decontamination on the total microbial burden on the spacecraft, shown in Table 2. It was reported earlier (1,2,3) that the total surface area of the assembled spacecraft was 500 square feet, but recalculation of the surface area proved it to be 526 square feet. Because the solar paddles and thermal blankets were treated differently (i.e., not decontaminated in the usual manner with isopropyl alcohol), the contamination on these surfaces after decontamination was added to the contamination detected on the spacecraft rather than averaged in. This resulted in a "worst-case" contamination level. The count shown for the thermal blankets were detected on

Table 3
Determination of the Total Microbial
Burden of the AIMP Flight
Spacecraft After Decontamination

Surface	Surface* Area (sq.ft.)	Viable Organisms
Spacecraft	194**	36,084
Solar paddles (4)	39	208,600
Thermal blankets (main and circular)	293	17,352
Average internal component burden	---	6,300
Total (sum)	526	268,336

**Includes area of internal components

*Surface areas determined by Mechanical Systems Branch

the exterior surface of the multilayered circular blanket only, after installation into the spacecraft. No contamination was detected on the main thermal blanket. Because laboratory tests showed that the thermal blankets were sterile, it was concluded that the surface of the circular blanket must have been contaminated during installation of the blanket in the spin facility, a nonclean room area. It required 10 minutes to install the circular blanket, and clean-room procedures were not observed.

Table 4 shows the results of decontamination or cleaning of the solar paddles with methyl ethyl ketone. There are several obvious reasons for the poor results obtained with MEK, but the most probable explanation is the highly volatile nature of the compound which makes it unsuitable as a decontaminating agent. The increase in the average burden after decontamination came from paddle "06," which yielded a count more than five times the highest count detected on the other three

Table 4
Decontamination of Solar Paddles
with Methyl Ethyl Ketone

Paddle	Date	Viable Organisms per Sq.Ft.	
		Before Decontamination	After Decontamination
06	6-29-66	2,088	16,200
07	6-29-66	1,332	936
08	6-29-66	1,296	1,116
09	6-29-66	468	3,204
02	6-29-66	3,276	---
Average/ft ²		1,692	5,364
Total Burden		82,400*	208,600**

*Total area (five paddles) = 48.7 ft²

**Total area (four paddles) = 39 ft²

paddles. This could have been caused by sampling of an area that was improperly cleaned; however, the reduction on paddles "07" and "08" was insignificant, and there was no reduction on paddle "09".

It was determined (4) that the internal component burden was a low of 1156 and a high of 11,444 for 32,612 components in the spacecraft. The average of these two figures (6300) was used as the average internal component burden. This number was added to the microbiological burden of the spacecraft (Table 3).

Table 5 shows the microbiological contamination in the air of the clean rooms which housed the AIMP spacecraft during the last stages of the assembly, as well as the number of personnel in the room during the sampling period. The counts in both rooms were extremely low which makes it difficult to generalize. However, the data indicate that counts were lowest in the downflow room which was not as heavily populated. Counts from the crossflow room were lower upstream than they were downstream. Because the spacecraft was transferred to the gantry on the twelfth day, the rooms were relatively unoccupied during the last 4 days except for cleaning. It is interesting to note that counts were lowest downstream in the crossflow room during the last 4 days; personnel appeared to have little or no influence on the upstream air.

Table 6 presents a microbiological profile of the air in two clean rooms during 2 typical work days. Samples were collected every hour for 7 hours. Lunch period occurred during the fourth hour on day one, whereas it occurred during the third hour on day two.

Table 7 lists the microbiological fallout on stainless steel strips over a 4-week period. Only aerobic vegetative cells were detected in the downflow room after 1 and 3 weeks of exposure. Aerobic spores detected after 3 weeks of exposure were the only microorganisms isolated upstream in the crossflow room. Aerobic and anaerobic vegetative cells, and aerobic and anaerobic spores, were detected after 1 and 3 weeks of exposure downstream in the crossflow room, respectively. From the center of the crossflow room, anaerobic spores and aerobic vegetative cells were detected after 1 and 3 weeks respectively.

DISCUSSION

Although NASA requires the sterilization of planetary-landing spacecraft, the sterilization requirement was removed from lunar

Table 5

Microbial Contamination in the Air of Laminar
Flow Clean Rooms Housing the AIMP Spacecraft
(viable particles/cubic foot/hour)

Day*	Downflow Room				Crossflow Room Upstream			
	AM		PM		AM		PM	
	Count	No. Personnel	Count	No. Personnel	Count	No. Personnel	Count	No. Personnel
1 (Fri)	0	1-2	.01	1-2	.05	6	.05	9
4 (Mon)	0	0-1	0	0-1	0	3-4	.01	6-8
5 (Tues)	0	0-2	0	0-2	0	6-8	.05	5-8
6 (Wed)	0	0-2	0	0-1	.01	3-5	0	4-8
7 (Thurs)	0	0	0	0	0	3-6	0	4-8
8 (Fri)	0	0-1	0	0	0	1-4	.01	2-7
11 (Mon)	0	0	0	0	0	0-2	0	1
12 (Tues)	0	0	0	0	0	1-3	.013	1
13 (Wed)	.013	0	0	0	0	1-2	0	1-2
14 (Thurs)	0	0	0	0	0	0-1	0	0-1
15 (Fri)	0	0	0	0	0	0-1	0	0
Average Viable Particles/Ft ³	.0012		.0009		.0055		.0120	

*June 3 - June 17, 1966.

Day*	Crossflow Room Downstream			
	AM		PM	
	Count	No. Personnel	Count	No. Personnel
1 (Fri)	0.2	6	---	---
4 (Mon)	.01	3-4	.05	6-8
5 (Tues)	.06	6-8	.08	5-8
6 (Wed)	0	3-5	.08	4-8
7 (Thurs)	0	3-6	.05	4-8
8 (Fri)	.05	1-4	.08	2-7
11 (Mon)	.016	0-2	0	1
12 (Tues)	.033	1-3	.033	1
13 (Wed)	0	1-2	0	1-2
14 (Thurs)	0	0-1	0	0-1
15 (Fri)	0	0-1	0	0
Average Viable Particles/Ft ³	.0335		.0348	

*June 3 - June 17, 1966.

Table 6

Microbial Contamination in the Air of Laminar Flow
Clean Rooms Over a 7-Hour Period

Hour	Viable Particles per Cubic Foot per Hour					
	Downflow Room			Crossflow Room Downstream		
	Day 1 (5-26-66)		Day 2* (5-19-66)	Day 1 (5-26-66)		Day 2* (5-19-66)
	Count	Per- sonnel	Count	Per- sonnel	Count	Per- sonnel
1	.033	0	---	---	.016	0-5
2	0	0-2	---	---	.033	4-8
3	0	0-1	.05	0	0	4
4	0	0	---	---	.016	0-3
5	0	0-2	.016	0	.066	0-8
6	0	0-2	0	0	.100	8
7	0	0-1	---	---	.050	4-7
Average Viable Particles per ft ³	.0047		.0220		.0401	
					.0218	

*Courtesy of Jerald Tritz, U.S. Public Health Service, Cape Kennedy, Florida

Table 7

Microbial Fallout on Stainless Steel*

Week of Exposure	Viable Organisms per Square Foot													
	Downflow Room				Crossflow Room									
					Upstream		Center				Downstream			
	Vege- tative Cells		Spores		Vege- tative Cells		Vege- tative Cells		Spores		Vege- tative Cells		Spores	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
1	57	0	0	0	0	0	0	0	0	57	29,016	57	0	0
2	0	0	0	0	-	-	-	-	-	-	-	-	-	-
3	120	0	0	0	0	0	122	0	0	0	0	0	360	360
4	-	-	-	-	0	0	0	0	0	0	0	0	0	0

*Courtesy of Jerald Tritz, Public Health Service, Cape Kennedy, Florida

landing spacecraft in 1963 and superseded by a new policy which stated that lunar spacecraft would be decontaminated to the best practical extent (5).

The objective of a decontamination program and assembly of spacecraft under a controlled environment, such as the one described in this report, is to reduce the number of contaminating microorganisms to the lowest possible level in order to prevent gross contamination of the lunar surface by terrestrial microorganisms. When sterilization of a spacecraft is the objective, a decontamination program will increase the probability of sterilizing the spacecraft during a given sterilization cycle.

Decontamination of the AIMP was important for several additional reasons:

- This was the first spacecraft which was completely decontaminated through all phases of assembly, and on which complete records were kept of the microbiological contamination.
- An applied working estimate of the microbial contamination of a typical spacecraft was obtained.
- Several problem areas, both biological and engineering were identified, such as decontamination of the solar paddles. (In future, these paddles can be manufactured to withstand dry heat sterilization, or an adequate decontaminating agent can be used to clean the solar cells.)
- The importance of the microbiologist-engineer team was recognized, as well as the necessity of close collaboration between the two disciplines and of a mutual understanding of various problems.

The early estimate (1) of 1×10^7 microorganisms on the surfaces of the AIMP spacecraft before decontamination and 2.8×10^5 microorganisms after decontamination was quite accurate. The actual total microbial burden determined was 3.72×10^6 before decontamination and 2.68×10^5 after decontamination.

The decontamination program was even more successful than the above numbers indicate, considering that the solar paddles alone yielded a microbial burden of 2.08×10^6 microorganisms for reasons

indicated above. Excluding the solar paddles, the total burden after decontamination was only 5.9×10^4 microorganisms, approximately a 2-log reduction. Die-off during orbit in space for 6 months or more may reduce the contamination further by as much as 2 logs (6). This would lower the contamination to an estimated 10^3 microorganisms at time of impact on the lunar surface.

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